ABSTRACT

Purpose: The cytochrome P-450 (CYP) and glutathione S-transferase (GST) enzyme systems modulate the carcinogenic effects of tobacco. Therefore, the expression of these enzymes may be in part responsible for the observed interindividual and inter-racial differences in the risk of development of squamous cell carcinoma of the head and neck (SCCHN). The first aim of this study was to evaluate the feasibility of measuring the expression of the CYP and GST in target tissue from the head and neck. The second aim was to compare the expression of CYPs 1A1, 2E1, and 3A4 in squamous epithelium from African-American and Caucasian pediatric patients. The third aim was to compare the expression of CYPs 1A1, 2E1, 3A4, and GST-\(\pi\) on the p16 expression in patients with SCCHN.

Experimental Design: The expression of CYP 1A1, 2E1, 3A4, GST-\(\pi\), and p16 was quantified by immunoblotting. Expression of CYPs 1A1, 2E1, and 3A4 was quantified in tissue from 160 pediatric patients undergoing tonsillectomy. Expression of CYPs 1A1, 2E1, 3A4, GST-\(\pi\), and p16 was determined in 46 resected SCCHN patients.

Results: Large interindividual variability in the expression of these enzymes was observed in the pediatric and adult populations. No significant difference was observed in CYP 1A1, 2E1, and 3A4 expression of Caucasian and African-American patients. There was no correlation between p16 and enzyme expression in patients with SCCHN.

Conclusion: Evaluation of CYP expression in the target tissue of interest is feasible. The clinical significance of CYPs and GST-\(\pi\) alterations in the risk of developing SCCHN will need to be investigated in larger trials.
Table 1  The biologic activity of CYP enzymes assayed in this study

<table>
<thead>
<tr>
<th>Activity</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP 1A1</td>
<td>Oxidative activation of polycyclic aromatic hydrocarbons and nitrosoamines forming keto-aldehydes</td>
</tr>
<tr>
<td>CYP 3A4</td>
<td>Oxidative activation of polycyclic aromatic hydrocarbons and nitrosoamines forming keto-aldehydes</td>
</tr>
<tr>
<td>CYP 2E1</td>
<td>Oxidative activation of polycyclic aromatic hydrocarbons and nitrosoamines forming keto-alcohols</td>
</tr>
</tbody>
</table>

Material and Methods

Study Population. Pediatric patients undergoing tonsillectomy and adult patients with SCCHN undergoing surgical excision were eligible for this study. All of the patients provided a signed informed consent in accordance with the Wayne State University Human Investigation Committee guidelines before enrolment on the study.

Tissue Collection. The pathological material was obtained fresh at time of surgery. The pathologist histologically examined tissue from patients with SCCHN. Tumor samples were obtained from unequivocal sites within the specimen. Normal tissue samples were obtained from morphologically normal adjacent tissue that was at least 1 cm away from the cancer site. All of the samples were covered with aluminum foil, sealed in plastic bags, coded, and stored at -70°C until analysis. Assays were done in batches of 10–20 samples and without knowledge of the sample source.

Western Blot Analysis (Immunoblotting). The minimum tissue weights sufficient for all of the intended analyses were 200 mg. Tissue samples were suspended in 2 ml of homogenizing buffer (pH 7.4) containing 50 mM Tris, 0.25 M sucrose, and 1 mM EDTA. The samples were then homogenized twice followed by centrifugation at 12,000 rpm for 30 min at 4°C. The supernatant was centrifuged at 45,000 rpm for 1 h at 4°C. The microsome pellet was suspended by sonication in 25 μl of microsomal storage buffer (50 mM Tris, 20% glycerol, and 1 mM EDTA). The concentration of proteins was determined by bicinchoninic acid assay using spectrophotometer and measured at an absorbance of 562 nm.

Approximately 20 μg of protein from each sample were loaded onto a 10% SDS-polyacrylamide gel along with three different concentrations of each of the known enzyme standards. The gels were run at 120 V for 90 min and subsequently transferred onto a nitrocellulose membrane. The membrane was blocked for 1 h at room temperature by PBS/0.05 Tween 20 containing 5% powdered milk. It was then treated with the primary antibody CYP 2E1, 1A1, or 3A4 (Gentest Corporation, Woburn, MA), GST-π (Oxford Biomedicals, Rochester Hills, MI), or p16 (Santa Cruz Biotechnology, Santa Cruz, CA) to the enzymes using optimized dilutions. Membranes were washed and treated with a secondary antibody (horseradish peroxidase conjugate). Protein bands were detected by enhanced chemiluminescent substrate. β-Actin expression was used to standardize the loading of the protein sample.

The intensity of the protein band was quantified by an image analysis system (Storm System; Molecular Dynamics, Piscataway, NJ). The intensity of the bands from the patient samples was determined from the standard linear curve encompassing the actual experimental measurements and corrected for the expression of β-actin in each sample. Enzyme levels were expressed in relative arbitrary units.

Statistical Methods. The enzyme distributions were highly skewed and non-normal, so nonparametric methods of analysis were used (12). Comparisons of enzyme levels between subgroups of patients were performed using the Kruskal-Wallis rank sum test (for three groups) or the Wilcoxon rank sum test (for two groups).

The strength of association between enzymes was assessed via a Spearman’s rank correlation. To control overall type I error, adjustment for multiple comparisons was performed using the Holm method (13).

RESULTS

Tonsillectomy Specimens. One-hundred and sixty pediatric patients undergoing tonsillectomy were enrolled on the study between June 1996 and August 1997 (Table 2). Fifty-three (34%) of the patients were African American. The distribution of patients by gender did not differ significantly over the race or the age groups. Similarly, the distribution of race did not differ significantly across the age groups. Wide interindividual variations in the expression of enzymes were noted. No significant relationship between the CYP 2E1, and either 1A1 (r = 0.133; P = 0.214) or 3A4 (r = 0.160; P = 0.142) enzymes was identified in this study indicating that the regulation of these isoenzymes is independent. A significant correlation was observed between CYP 1A1 and 3A4 (r = 0.224; P = 0.008) even after adjustment by the Holm procedure for the type I error of 6 correlations. Expression of CYP enzymes 1A1, 2E1, and 3A4 were compared by age group, gender, and race (Table 3). There were no significant differences by gender, age, or race in the level of CYP 1A1, 2E1, and 3A4.

Table 2  The characteristics of the 160 pediatric patients undergoing tonsillectomy and the 46 patients with SCCHN enrolled on this study

<table>
<thead>
<tr>
<th>Tonsillectomy group</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>67</td>
<td>42</td>
</tr>
<tr>
<td>5–6 years</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>&gt;7 years</td>
<td>43</td>
<td>28</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>53</td>
<td>34</td>
</tr>
<tr>
<td>Caucasian</td>
<td>80</td>
<td>52</td>
</tr>
<tr>
<td>Other</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>99</td>
<td>62</td>
</tr>
<tr>
<td>Female</td>
<td>61</td>
<td>38</td>
</tr>
<tr>
<td>SCCHN group</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 years (mean)</td>
<td>28</td>
<td>60</td>
</tr>
<tr>
<td>60 years (mean)</td>
<td>18</td>
<td>40</td>
</tr>
</tbody>
</table>

* SCCHN, squamous cell carcinoma of the head and neck.
The SCCHN Specimens. Forty-six patients were enrolled on the study. CYP 1A1, 2E1, 3A4, and GST-π levels of expression did not differ significantly by gender (Table 4). Fig. 1 demonstrates a representative immunoblot of the four enzymes that were studied. Fig. 2 demonstrates a representative immunoblot of p16 expression. Wide interindividual variations in the expression of enzymes were noted. No significant correlation between the CYP 2E1 and either 1A1 (r = 0.14) or 3A4 (r = 0.21) enzymes was identified in this study indicating that the regulation of these isoenzymes is independent. The strongest correlation observed was between CYP 1A1 and 3A4 (r = 0.406). There were no significant correlations between p16 and the level of expression of CYP 1A1, 2E1, 3A4, and GST-π. The strongest correlation was a negative one between p16 and CYP 1A1 (r = -0.164).

A comparison of the expression of GST-π, CYP 3A4, 1A1, and 2E1 between histologically normal and malignant tissue from 16 patients with SCCHN is shown in Fig. 3. A significantly higher expression of CYP 3A4 and 1A1 was observed in normal tissue, even after adjustment for the type I error of a set of four comparisons. No difference in expression of CYP 2E1 and GST-π was present.

DISCUSSION

Polycyclic aromatic hydrocarbons, aromatic amines, and nitroso compounds are among the carcinogens that have been identified in tobacco smoke (14). The CYP enzymes oxidize these compounds yielding reactive epoxide intermediates, which can covalently bind and alter DNA structure (15). GST enzymes catalyze glutathione conjugation of these intermediates thereby decreasing their DNA damaging effects (16). Variations in the expression of CYPs and GST could potentially explain the observed difference in vulnerability to the carcinogenic effects.
of tobacco. Previous studies have demonstrated the expression of CYPs and GST in normal squamous epithelium of the head and neck (9, 10, 17). CYP and GST expression varies in different tissues (18, 19). Tissue metabolism of carcinogens by these locally expressed enzymes may be a more important determinant of carcinogenesis than metabolism in the more distant organs such as the liver. Several studies assessed the risk of aromatic amines and cancer. Cancer Causes and Practice of Oncology 1. Philadelphia: Lippincott Williams & Wilkins, 1993. p. 179

REFERENCES


Enzyme Expression in SCCHN


Cytochrome P450 and Glutathione Transferase Expression in Squamous Cell Cancer


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